Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 6, Issue, 08, pp.7724-7730, August, 2014

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

IMPACT OF PHYTOPESTICIDE NEEM GOLD ON OVARIAN TISSUES IN *ODONTOPUS VARICORNIS* (Dist.) (HEMIPTERA : PYRRHOCORIDAE).

***Ramesh kumar, T. and Sethuraman, S.**

Department of Zoology, Annamalai University, Annamalai Nagar-608002, Tamilnadu, India

ARTICLE INFO

Article History:
Received 08th May, 2014
Received in revised form
10th June, 2014
Accepted 15th July, 2014
Published online 06th August, 2014

Key words:
Germarium, Vitellarium, Gonopore, Nutritive cord, Trophic core, Follicular epithelium.

ABSTRACT

The dynamic aspects of insect reproduction has received more attention in recent years. Insect reproduction is an essential physiological process from the view point of propagation due to the fact that, its processes are so intimately associated with other systems and are controlled by both intrinsic and extrinsic factors. The male and female reproductive system generally consists of paired gonads connected to a median duct leading to the gonopore. Insect ovaries are composed of several ovarian tubes termed ovarioles. The classification of the ovary type is essentially based on the general architecture of the ovarioles. In panoistic ovarioles all germ cell becomes oocyte and consequently egg cells. In meroistic ovaries divisions of ogonial cells are fallowed by incomplete cytokinesis. In the phytopesticide neem gold treated ovaries (25ppm median lethal concentration) of *Odontopus varicornis* shows drastic histopathological changes in germarium, follicular epithelium, trophocytes and oocytes. The application of neem gold results in a significant reduction in the rate of cell membrane, chromatin clumbing in the nuclei of zone 1, zone 2 & zone 3. The follicular epithelium exhibits a shrunken and folded appearance with damaged trophic core and nutritive cords.

INTRODUCTION

Among invertebrates, especially in arthropods insects have received much attention in the study of reproductive biology. The high biotic potential of insects makes the reproductive process a subject of importance in applied entomology. In recent years, the functional aspects of insect reproduction was attracted more attention (Adiyodi and Adiyodi, 1974). The great complexity of morphology and physiology of insects renders them an interesting group of investigation. Individually each insects exhibits an unique way of reproduction. Reproductive physiology of female insect is a complicated phenomenon and it deals with the structure and functions of various tissue components. Insects reproduction is an essential physiological process from the point of view propagation and it was received relatively little direct attention perhaps due to the fact that, its processes are so intimately by both intrinsic and extrinsic factors. Insects by virtue of their economic importance have been the object of study for a long time in particular from the point of view of pest control and management. The common methods employed to compact insects pests include the use of a variety of approaches *viz*; the application of insecticide, chemosterilants, hormones, biological control, cultural practices and mechanical devices. In recent years the dynamic aspects of insects reproduction has received more attention (Chapman, 1972). In insects the polytrophic ovaries, ponoistic ovaries and telotrophic ovaries have revealed that the follicle cells and the trophic or nurse cells, play an importance role in transferring the nutritive materials for the development of oocytes (Highnam, 1962; Madhavan, 1964; Agarwal, 1967; Matsuzaaki, 1972; Kamalakannan, 1977). The origin of protein yolk originates in the oocyte by the transformation of nuclear emission particles (Anderson, 1964). The nuclear buds get extruded through the nuclear membrane into the cytoplasm and form protein yolk with mitochondria. The ribonucleus protein supplied by the trophocytes through the nutritive cords seem to get involved in the formation of protein yolk. The germarium of the telotrophic ovary of *Odontopus varicornis* is composed of oogonial cells and the cells of the trophic tissue. The germarium has a central core of cytoplasm surrounded by densely packed cells of the trophic tissue. The trophic tissue consists of nurse cells or trophocytes. The entire germarium is divided into three regions. The follicle cells and the trophocytes play a significant role in contributing nutritive substances to the developing of prefollicular tissue differentiates into a follicular epithelium around the oocytes when they approach the vitellarium. Vitellarium is a large part of the ovariole and is characterized by the large number of developing oocytes arranged in a linear order. Each developing oocyte is enclosed in a follicle of the epithelial cells, and the developing oocytes are seprated from each other by inter follicular tissue. Vitellogenisis, a complicated process of synthesis and deposition of yolk that take place during oogenesis. It is an dynamic aspects in insect reproductive physiology (Adiyodi and Adiyodi, 1974).

****Corresponding author: Ramesh kumar, T.***
Department of Zoology, Annamalai University, Annamalai Nagar-608002, Tamilnadu, India.

India is endowed with rich tropical flora, which are considered store houses of plant production chemicals. It has been estimated that only 5-15 percent of the existing plant species have been surveyed for biologically active compounds. Pesticides of plant origin have the additional advantage over the traditional chemical pesticides, of quick biodegradability, safety to non target organisms and non- persistence. The Indian neem tree has been found to be a promising source of natural pesticides with the above qualities. Neem has a history of use primary against household and storage pests and to a limited extent against crop pests in Indian subcontinent (Saxena, 1989). Neem oil based antifeedant / repellant has been prepared to control sucking, scrapping and leaf eating caterpillars on paddy, cotton and pulses (Schmutterer, 1990). Azadirachtin enriched neem extract is used in the management of human and animal pests. In the last decades plant extracts with insecticidal properties have evoked a substantial scientific and practical interest as a natural means of insect and mite pest control in sustainable agriculture. Increasing use various pesticides for arresting the damage caused by pest has brought in its wake certain complications as pesticides pollute the soil, water, atmosphere and kills the animals. Nowadays the farmers use a large number of pesticides and insecticides to control the pests, damaging crop and insects respectively. The poor knowledge about the pesticides effect, pesticide application method among farmers is one of the main reasons for pollution. Extract of the Indian neem tree *Azadirachtica indica* are gaining prominence, the foremost biotoxical insecticides (Prabhakar et al, 1968 and Stone, 1992). Azadirachtin has antifeedant- antiovipostional, growth disrupting and fecundity reducing properties on different insects (Schmutterer, 1990). Phytochemicals can be extracted from either whole plant or specific parts of the plant, depending on the activity of the derivatives, some plants accumulate bioactive chemicals differentially in the various parts of the plants such as leaves, fruits, flower, root and bark. Some phytochemicals act as general toxicant to all life stages of mosquitoes and other vectors (Singh and Thangavelu, 1986).

The plant sucking insect *Odontopus varicornis* which belongs to the order Hemiptera, family Pyrrhocordae most of the Hemipteran insects are sap feeders or suckers and thus regarded as serious pest in arthropods. Insect have received much attention in the study of reproductive biology. Insects ovaries are composed of several ovarian tubes termed ovarioles. The classification of the ovary type is essentially based on the general architecture of the ovariole but most of all on the analysis of the ultimate fate of the developing germ cells (Buning, 1994 & 1998). In *Odontopus varicornis* there are two ovaries, each consists of five telotrophic type of ovarioles. The two lateral oviduct leading from each ovary unite to form the median common oviduct. The common oviduct runs backwards and opens to the exterior. There is a spermatheca attached at the posterior part of the common oviduct. The ovarioles of the ovary are connected together anteriorly by their terminal filaments and posteriorly by their pedicels.

MATERIALS AND METHODS

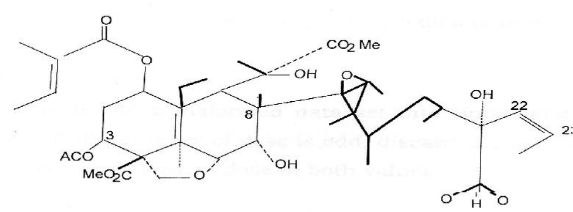
Adult female insects were selected from the cage. The adult control and treated insects were dissected using insect ringer solution (Ephrussi and Beadle, 1936). The removed ovary were fixed in aqueous Bouins fixative. After 24 hrs of fixation, the tissue was processed for dehydration using ascending grades of alcohol (Gurr, 1958). The tissue was gross stained in 70 percent aqueous eosin to facilitate orientation during embedding. the tissue after dehydration in absolute alcohol and acetone were cut at 6µ thickness were deparaffinised using descending grades of alcohol and stained with haematoxylin and counter stained with aqueous eosin for microphotographs.

Table 1. Nature of Insecticide

S.NO	NATURE OF INSECTICIDE
01	Plant name: Margosa (*Azadirechta indica* A.Juss)
02	Family: Meliacea
03	Part of the plant used: Bark
04	Structure and properties: Azardirechtin
05	Trade name: Neem Gold
06	Sample description: Commercial Grade
07	Chemical name: Tetrapo Triterpenoid
08	Molecular formula: $C_{35}H_{44}O_{16}$

Table 2. **Chemical Composition**

S.NO.	CHEMICAL COMPOSITION
01	Azadirectin: 0.03% w/w
02	Neem oil: 90.57%
03	Hydroxyl EL:0.05.00% w/w
04	Epichlordrin: 00.50% w/w
05	Aromex: 0.03.90% w/w
06	(SPIC) Southern Petrochemicals. Industries Corporation (Ltd) Chennai, Tamilnadu
07	Appearance: White oily
08	Solubility: Water miscible
09	Principle use: Insecticide
10	Molecular structure



RESULTS AND DICUSSION

Impact of neem gold on germarium

In *Odontopus varicornis* The germarium of the telotrophic ovary is composed of oogonial cells and the cells of trophic tissue. The germarium has a central core of cytoplasm surrounded by densly packed cells of the trophic tissue. The trophic tissue consists of nurse cells or trophocytes. The entire germarium is divided into three regions i.e. zone1, zone 2 and zone 3 (Fig.1).

The anterior part of the germarium is designated as zone I, is composed of cells showing intense mitotic activity. Each cell with its distinct cell boundary contains spherical nucleus with a large centrally located nucleolus surrounded by coarse chromatin granules (Fig. 2)

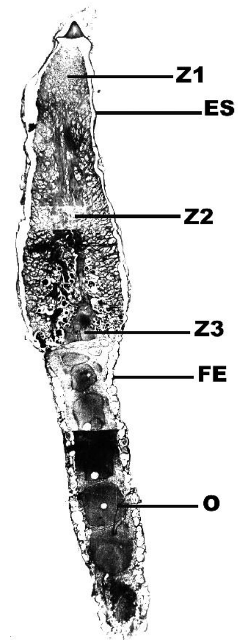


Fig. 1. Longitudinal section of the ovariole of an adult control insect. ES- epithelial sheath; FE- follicular epithelium; Z1- zone 1; Z2- zone 2; Z3- zone 3; O- oocyte

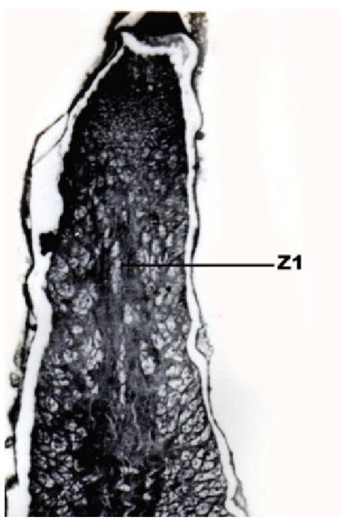


Fig. 1. Longitudinal section of the ovariole of dimethoate treated insect. Z1- zone 1

The middle part of the germarium designated as zone 2 is composed of the cells of the trophic tissue and they seems to differ from those of the zone 1. The cells are larger than the cells of zone 1 and their cell boundaries still remain indistinct. Each nucleus is characterized by chromatin substance in the form of granules. The posterior part of the germarium designated as zone 3 is characterized by the appearance of several aggregations of nuclei which are found in different stages of fusion. The completely fused nuclei appear to be larger in size each possessing a large nucleolus and distinct chromatin granules. Mitotic activity is not encountered in the cells of the zone 2 and 3 (Fig.3 and 4).

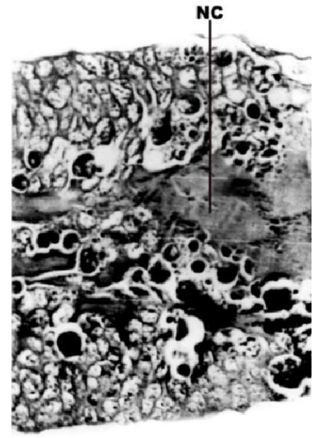


Fig. 3. Longitudinal section of the anterior region of the germarium of control insect

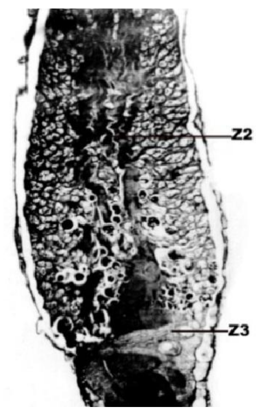


Fig. 4. Longitudinal section of the posterior region of the germarium of control insect

NC- nutritive cord; Z2- zone 2; Z3- zone 3.

In the neem gold treated (25ppm median lethal concentration) germarium of zone 1 shows signs of cytoplasmic vacuolization and disintegration (Fig. 5).

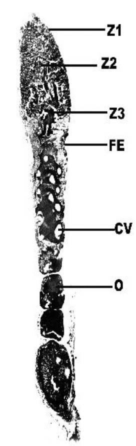


Fig. 5. Longitudinal section of germarium of control insect. FE- follicular epithelium; Z1- zone 1; Z2- zone 2; Z3- zone 3; O- oocyte; CV- cytoplasmic vacuolization

The nuclei of these cells shows chromatin clumping which is intensely stained with haematoxylin. The cell boundaries are not distinct. Mitotic activity could not be identified, perhaps due to the chromatin clumbing. There is no marked changes in the size of the cells although their arrangement is somewhat disturbed (Fig. 6)

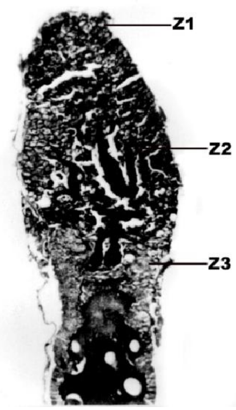


Fig. 6. Longitudinal section of the germarium of dimethoate treated insect. Z1- zone 1; Z2- zone 2; Z3- zone 3;

The cells of zone 2 exhibit histopathological changes similar to those of zone1. further the flow of the cytoplasm from the trophic tissue to the trophic core is damaged in several regions (fig. 5). The cells of the trophic tissue of zone 3 indicate cytoplasmic vacuolization and chromatin clumping. The nutritive cords of the trophic core have drastically reduced in size and exhibit several foldings (fig. 6). The trophic core appears to have been broken into few regions of the cytoplasmic masses. All these changes have resulted in the reduction in the size of the germarium. In *Odontopus varicornis* the trophic tissue occupies two third of the germarium which is divisible into zone 1, zone 2 and zone 3 and the cells are closely packed around the central core of cytoplasm, further the nuclei of zone 1, indicating mitotic activities from clusters of nuclei in zone 2 and latter undergo fusion in zone 3 of the germarium (Fig. 7 and 8).

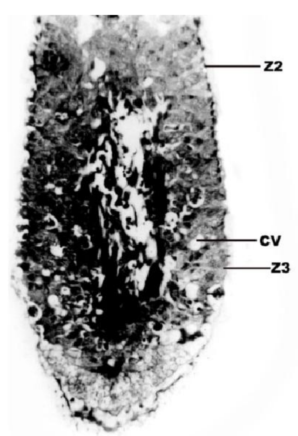


Fig. 7. Longitudinal section of the anterior region of the germarium of dimethoate treated insect

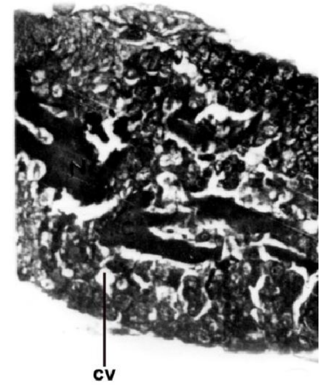


Fig. 8. Longitudinal section of the posterior region of the germarium of dimethoate treated insect. Z2- zone 2; Z3- zone 3; CV- cytoplasmic vacuolization

This general organization and the behavior of the nuclei of the trophic tissue appears to be similar to those reported for *Acanthocephla bicoloripes* (Schradder and Leuchlenberger, 1952). The zone 3 trophic nuclei of *Odontopus varicornis* have been observed to undergo remarkable cytological changes leading to their fusion developing into large nuclei in close association with the trophic core. This circumstantial evidence suggests the possibility of the transference of nuclear material from the trophocytes to the trophic core to the supplies to the growing oocyte through the nutritive cords. This inference gains support from the histological evidences indicating an accumulation of nuclear material at the junction between the trophic core and the nutritive cords, in *Chrysocoris purpureus* (Madhavan, 1964).

Impact of Neem Gold on Vitellarium

Histological observations on the ovary of *Odontopus varicornis* have revealed that the trophocytes of zone 3 unlike those of zone 1 and zone 2 have distinct cell boundaries on the other hand treatment with neem gold has resulted on the destruction of cell boundaries in the cells. It is evident from this observation that neem gold seems to affect structural components of the cell membrane. In treated *Odontopus varicornis* cause cytoplasmic vacuolization, degeneration of trophocytes and chromatin clumping in nuclei of their cells (Fig. 9 and 10). Many chemosterilants such as Hempa, Metepa, Tepa, Apholate, Colchicines and Thiourea have been reported the same changes in *Drosophila melanogaster* (Jacob, 1958) housefly (Mitlin and Broodr, 1958). *Heliotheis virescens* (Soto and Growes, 1967), *Acanthocelids obtectus* (Ondrack and Matolin, 1971).

Impact of Neem Gold on Follicular Epithelium

The follicle cells and the trophocytes play a significant role in contributing nutritive substances to the developing oocytes during the development the pre follicular tissue differentiates into follicular epithelium around the oocytes when they approach the vitellarium. The follicular epithelium surrounding the alpha oocyte is composed of a single layer of columnar follicle cells. The cytoplasm of this follicle cells is dense and it possesses a large nucleus with a nucleolus (Fig. 11).

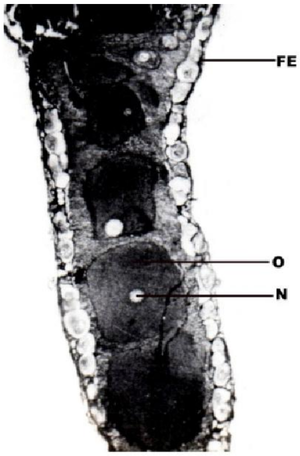


Fig. 9. Longitudinal section of the germarium of dimethoate treated insect

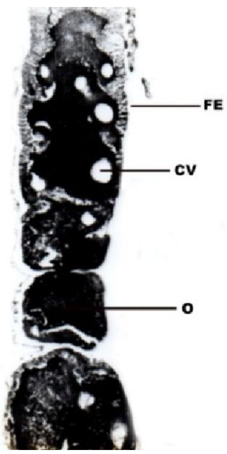


Fig.10. Longitudinal section of the vitellarium of control insect. CV- cytoplasmic vacuolization FE- follicular epithelium; O- oocyte; N- nucleus

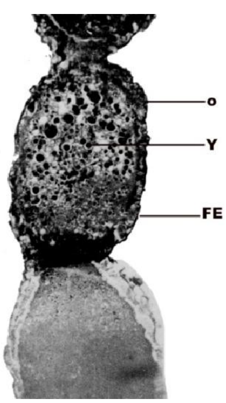


Fig.11. Longitudinal section of the vitellarium of dimethoate treated insect. Y- yolk spheres; FE- follicular epithelium; O- oocyte

The follicle cells which are trapped between the descending oocytes constitute the interfollicular plug. These cells do not differentiate further and they exhibit occasional mitotic activity (Fig. 12).

Fig.12. Longitudinal section of the vitellarium of control insect. Y- yolk spheres; O- oocyte

Treatment with phytopesticide, neem gold (25ppm median lethal concentration) affects the normal structure of the follicular tissue appears to have been disorganized. The epithelium does not indicate distinct intercellular spaces. The nuclei of the follicle cells surrounding the developing the oocytes showed signs of nuclear pynocsis and chromatin clumbing. The present observation on *Odontopus varicornis* have revealed that the follicle cells are affected by phytopesticide neem gold caused cytoplasmic vacuolization, chromatin, clumping, pycnosis of the nuclei and disintegration of cells (Fig. 13 and 14).

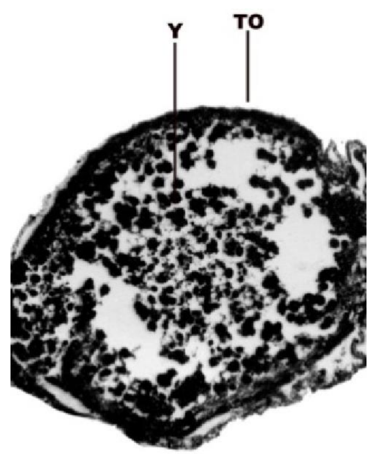


Fig.13. Longitudinal section of the fully mature ovariole of control insect

The treatment with some chemosterilants have been shown to produce several damage to follicular cells in *Musca domestica* (Morgan and Lebreque, 1962 and 1964). Application of colchicines has produced similar effects in *Chrysomyia megacephala* (Sukula and Singh, 1982).

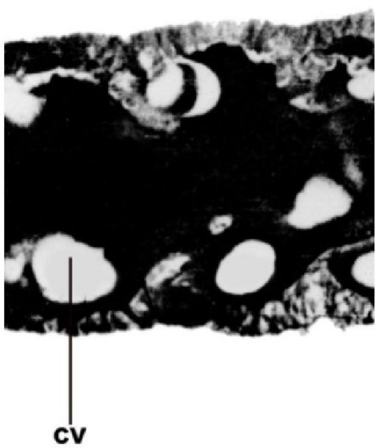


Fig.14. cross section of the oocyte of control insect. Y- yolk spheres; CV- cytoplasmic vacuolization

It may be inferred on the basis of present observations and those of other report that the inhibition of ovarian development in insects treated with neem gold is to be correlated with the histopathological changes of the follicular cells which seems to fail to transport the raw material essential for the development of the oocyte vitellogenesis. Vitellogenesis, a complicated process of synthesis and deposition of yolk that takes place during oogenesis. It is dynamic aspect of insect reproductive physiology and has received more attention in recent years (Chapman, 1972). The oocytes are designated as delta, gamma, beta and alpha oocytes (Bonhag, 1955a). About fourteen oocytes are found in the vitellarium is a chronological sequence, the oldest being situated near the pedicle. The delta oocytes are surrounded by pre follicular tissue. The nucleus is centrally placed which is stained intensively with haematoxylin (Fig. 15).

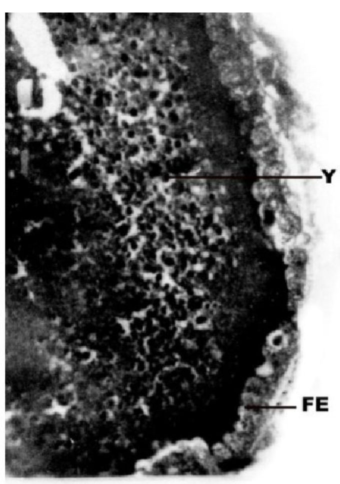


Fig.15. Longitudinal section of the oocyte of dimethoate treated insect. Y- yolk spheres; FE- follicular epithelium

In beta oocyte the yolk particles are distinct in the pheripheral ooplasm of the beta oocyte. The alpha oocytes are densely packed with yolk granules (Fig. 16).

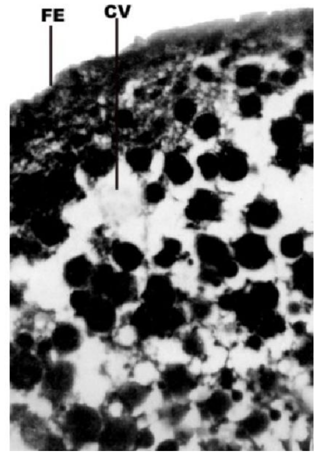


Fig.16. Longitudinal section of the oocyte of dimethoate treated insect

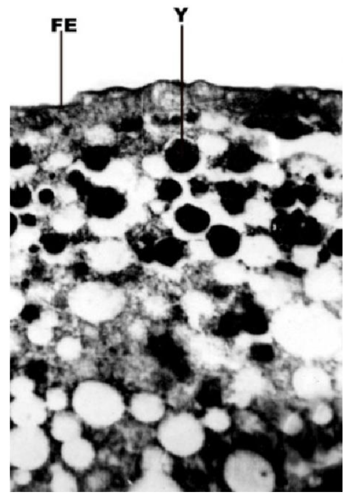


Fig.17. section of the portion of the oocyte control insect.Y- yolk spheres; FE- follicular epithelium; Y- yolk spheres; CV- cytoplasmic vacuolization

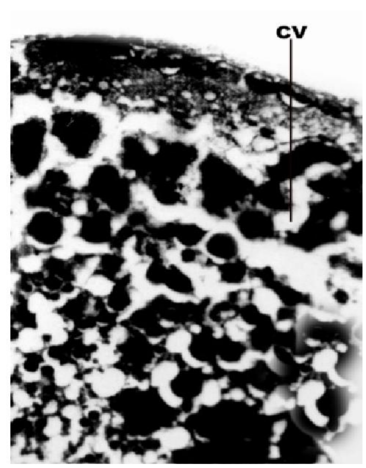


Fig.18. section of the portion of the oocyte dimethoate treated insect. CV- cytoplasmic vacuolization

The vitellarium of the treated ovaries the oocytes exhibites cytoplasmic vacuolizations (Fig. 17). The germinal vesicle of the oocyte appears to be elliptical in shape and shows chromatin clumping. The fully mature alpha oocyte of the neem gold treated insects shows a significant reduction in the concentration of yolk spheres. Neem gold treated insects results in ooplasmic vacuolization, chromatin clumping, pycnosis of the nuclei reduction in size of the oocyte and the concentration of yolk spheres. (Fig. 18).

It is evident from this observation that the ovarian development is inhibited probably due to the action of neem gold used in this investigation. The present findings correlated with those of (Schwartz, 19650; Dutt and Chandan Mukharji, 1970; and Sukumar *et.al*, 1973). Who have shown a general reduction in the size of the ovarioles respectively in *Hippelatus pusio, Apion corchori and Dysdercus cingulatus* due to chemosterilants such as apholate, tepa and metepa. In *Dysdercus cingulatus* treatment with certain plant arrest of oocyte development. It is well established that oocyte development and vitellarium in insect are under the regulation of neuroendocrine system. (Engelmann, 1970; Chapman, 1972). It is evident from these reports that inhibition of ovarian development and vitellogenesis in *Odontopus varicornis* appears which is not only due to the histopathological changes of the ovarian tissues but also due to the impact of phytopesticide in the neuroendocrine system.

REFERENCES

Adiyodi, K.G. and Adiyodi, R.G. 1974. Control mechanism in cockroach reproduction. *J. Sci. Inds. Res., India*. 33: 343 - 358.

Aggarval, S.K. 1967. Morphological and histochemical studies on oogenesis in *Callosobruchus analis* Fabr. (Bruchidae: Coleopteran). *J. Morph*., 122: 19-34.

Anderson, E. 1964. Oocyte differentiation and vitellogenesis in the cockroach *Periplaneta Americana*. *J. Cell. Boil*., 20 (1): 131-155.

Bonhag, P.F. 1955a. Histochemical studies of the ovarian nurse tissue and oocyte of the Milkweed bug, *Oncopeltus fasciatus* (Dallas). I. Cytology, Nucleic acids and Carobohydrates. *J. Morph*., 96(2): 283-312.

Buning, J. 1994. The insect ovary. Ultrastructure, previtellogenic growth and evolution. Chapman & Hall, London.

Buning, J. 1998. The ovariole: structure, type and phylogeny. In: Locke, M., Harrison, H. (Eds): microscopic anatomy of invertebrates. Viley-Liss, New York, 1c: 897-932.

Chapman, R.F. 1972. Insect structure and function. The English universities press Ltd., London. pp. 3-749.

Dutt, N. and Chandan Mukherjee 1970. Effects of chemosterilants on the reproductive organs of the jute Stem Weevil, *Apion corchorimarsh*. (Col: Apionidae). *Indian J. Ent*., 32(4).342-349.

Engelmann, F. 1968. Hormonal control of egg maturation, In: The physiology of insect reproduction. International series of monographs in pure and applied biology, 44. General Ed. G.A. Kerkut. pp.143-149.

Ephrussi, B. and Beadle, G.W. 1936. A technique of transplantation for drosophila. Amer. Nat., 70:218-225.

Gurr, E. 1958. Methods of analytical histology and histochemistry. Leonard hill (Books Ltd.), London.

Highnam, K.G. 1962a. Neurosecretory control of ovarian development in schistocerca oregaria. *Quart. J. Micr.Sci*., 102: 27-38.

Jacob, J. 1958. A study of colchicines induced sterility in the female Fruitfly *Drosophila melanogaster*, Growth., 22: 17-19.

Kamalakannan, D.R. 1977. Studies on ovarian development and neuroendocrinre system in *Odontopus varicornis* (Dist.) Ph.D. Thesis, Annamalai University.

Madhavan, K. 1964. Studies on the corpus allatum and ovary in *Chrysocoris purpureus* (Westw). Ph.D. Thesis, Annamalai University.

Matsuzaki, M. 1972. Oogenesis in adult net-spinning caddisfly *Parastenopsyche sauteri* (Trichoptera: Steropsychidae) as revealed by electron microscopic observation. Sci. Rep. Fukushima, Univ., 22: 27-40.

Mitlin, N. and Baroodr, A.M.1958. The effect of some biologically active compounds on growth of house-fly ovaries *.j. Econ.Ent*., 58: 384-385.

Morgan, P.B. and Labrecque, G.C. 1962. The effect of apholate on the ovarian development of house-fly *Musca domestica*. *J. Econ.Ent*., 55(5): 626-628.

Morgan, P.B. and Labrecque, G.C. 1964. Effect of tepa and metepa on ovarian development of house flies. J. Econ. Ent., 57: 896-899.

Ondracek, J. and Matolin, S. 1971. Sterilizing effect of tepa on the bean beetle, *Acanthosceliads obtectus* say (Coleopteran) *Acta. Ent. Bohemoslov*., 68: 209-215.

Prabhakar, N., Condrict, D.C., Kishaba, A.N. and Negar Dirk, D.E. 1968. *J. Beon. Entomol*., 79:39-49.

Schrader, F. and Leuchtenberger, C. 1952. The origin of certain nutritive substances in the eggs of Hemiptera. *Exptl. Cell Res*., 3(1): 136-146.

Schwartz, P.H. 1965. Effects of apholate, metpa, tepa on the reproductive tissue of hippelatus Pusio. Loew. *J. Invert.Pathol*., 7(2): 148-151.

Shukla, G.S. and Singh, R.N. 1982. Histological effect of chemosterilant (Colchicina) on the ovarian tissues of *Chrysomyia megacephala* fabr. (Dipter: Calliphoridae). *J. Ani. Morphol. Physiol*., 29(1, 2): 262-264.

Singh, R.N. and Thangavelu, K. 1968. Influence of neem compound on the growth and development of immature forms of Cercifly *Blepharia zebina* (Kalk) (Dipteral: Technidae), Pestology, 31.

Soto, P.E. and Growes, J.B. 1967. Chemosterilization of bollworms and tobacco bud worms. *J. Econ. Ent*., 60: 550-552.

Stone, C. 1992. Science (Washington), 255:1070-1071.

Sukumar, K. and Naidu, M.B. 1973. Inhibition of ovarian growth by tepa in dysdercus cingulatus. *J. Econ. Ent*., 66(1): 20-22.

****